



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> SERTOLI CELLS AS NEURORECOVERY INDUCING CELLS FOR NEURODEGENERATIVE DISORDERS <b>(57) Abstract</b>  A method of generating <i>in situ</i> trophic factor production by transplanting Sertoli cells into a tissue in need of trophic factors of a mammal, the cells creating trophic factors <i>in situ</i> .		

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**SERTOLI CELLS AS NEURORECOVERY INDUCING CELLS  
FOR NEURODEGENERATIVE DISORDERS**

**TECHNICAL FIELD**

5                   The present invention generally  
relates to cell transplantation and specifically to  
a method of transplanting cells which, following  
transplantation into the central nervous system  
(CNS), ameliorates the behavioral and functional  
10 deficits associated with neurological and  
neurodegenerative disorders.

**BACKGROUND OF THE INVENTION**

                  In treating disease it is often useful to  
15 treat tissue locally, rather than systemically,  
with trophic factors, particularly areas of tissue  
damage as for example in wound healing.

                  As a further example, transplantation of  
neural tissue into the mammalian central nervous  
20 system (CNS) is becoming an alternative treatment  
for neurological and neurodegenerative disorders  
including epilepsy, stroke, Huntington's diseases,  
head injury, spinal injury, pain, Parkinson's  
disease, myelin deficiencies, neuromuscular  
25 disorders, neurological pain, amyotrophic lateral  
sclerosis, Alzheimer's disease, and affective  
disorders of the brain. Preclinical and clinical

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data indicate that transplanted cells (the graft) used in cell transplantation protocols for these types of neurodegenerative diseases survive and integrate with the host tissue, and provides  
5 functional recovery. (Sanberg et al., 1994).

The primary source for these grafts has been the fetus. For example, fetal ventral mesencephalic tissue has been demonstrated to be a viable graft source in Parkinson's disease.  
10 (Lindvall et al., 1990; Bjorklund, 1992). Likewise, fetal striatal tissue has been utilized successfully as graft material in Huntington's disease. (Isacson et al., 1986; Sanberg et al., 1994).

15 Neurologically dysfunctional animals have been transplanted with non-fetal cells and non-neuronal cells/tissue. For example, chromaffin cells from adult donors have been used in the treatment of Parkinson's disease. The major  
20 advantage of this type of transplantation protocol is that the graft source is not a fetal source and, thereby, circumvents the ethical and logistical problems associated with acquiring fetal tissue. Utilizing the chromaffin cell protocol,  
25 normalization of behavior is observed. However, the functional recovery of this behavior is

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temporary and the animals revert to their pre-transplantation status (Bjorklund and Stenevi, 1985; Lindvall et al., 1987). The inability of this type of treatment protocol to maintain normal behavioral activity in animals in the Parkinson's disease model renders clinical application of this protocol as well as other treatment therapies premature.

Administration of growth factors as a means of treating neurological and neurodegenerative diseases has been contemplated in the art. However, delivering these agents to the brain is fraught with great difficulties that have yet to be successfully overcome. Generally, these agents cannot be administered systemically and infusion into the brain is an impractical and imperfect solution. Engineering cells to deliver specific, single trophic factors when implanted in the brain has been suggested, but stable transfection and survival of the cells when implanted in the brain continues to be problematic. Additionally, it is becoming increasingly recognized that multiple trophic factors acting in concert are likely to be necessary for the successful treatment of neurological and neurodegenerative conditions.

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Long term maintenance of functional recovery has been observed in a diabetic animal model utilizing a novel transplantation treatment protocol utilizing isolated islet cells and Sertoli  
5 cells. It is clear that the efficacy of the treatment is due to the presence of the Sertoli cells, in part, due to their known immunosuppressive secretory factor. (Selawry and Cameron, 1993; Cameron et al., 1990). Sertoli  
10 cells are also known to secrete a number of important trophic growth factors.

Accordingly, it would be desirable to utilize Sertoli cells alone as a source for diseases where growth and trophic factor support of  
15 damaged tissue is useful. Examples include, wound healing and neurological disorders including neurodegenerative disorders. The Sertoli cells can be used to function as an *in situ* factory for trophic factors to thereby hasten wound healing and  
20 to ameliorate functional and behavioral deficits associated with neurological and neurodegenerative disorders.

### SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a method of generating *in situ* trophic factor production by transplanting Sertoli  
5 cells into a mammal, the cells secreting trophic factors *in situ*.

### BRIEF DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention  
10 will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is a graph showing the results  
15 of apomorphine-induced rotational behavior, animals from both groups exhibited >7 rotations per minute or, at least, a total of 210 rotations for 30 minutes (contralateral to the lesion) when challenged with apomorphine pre-transplant, at  
20 post-transplant periods, animals receiving media alone continued to display significant rotations, in contrast, animals receiving the Sertoli cells had a marked reductions (more than 60%) in their rotational behavior across the post-transplant  
25 periods;

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Figure 2 is a graph showing biased swing behavior, animals from both groups displayed >80% biased swing activity (contralateral to the lesion) as revealed by the elevated body swing test, at  
5 post-transplant periods, animals receiving the media alone continued to display significant biased swing activity, in contrast, animals receiving the Sertoli cells did not exhibit any biased swing behavior across the post-transplant periods;

10 Figure 3A-C are light micrographs illustrating cells from the ventral mesencephalon of fetal rats (VM) isolated and cultured for seven days in control medium (CM) or Sertoli cell pre-conditioned medium (SCM) and photographed with  
15 darkfield, interference contrast optics, wherein (A) depicts VM cells incubated in CM showing no evidence of stimulation or differentiation, (B) depicts VM cells incubated in SCM appearing highly stimulated, and (C) at higher magnification,  
20 depicts VM cells incubated in SCM exhibiting neurite outgrowth as a result of Sertoli secreted trophic factors;



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Figure 4A-B are electron micrographs illustrating (A) the striatum of the brain showing the penetration tract (arrows) and the site of Sertoli cell transplantation, and (B) shows the boxed area in (A) at higher magnification, with higher resolution, Sertoli cells (arrows) are easily identified because of the 1 $\mu$  latex bead inclusions which were loaded into the cells prior to transplantation; and

Figure 5A-B are two light micrographs illustrating grafted Sertoli cells *in situ* labeled with a florescent tag (DiI) prior to their transplantation into the striatum of the brain wherein (A) depicts viable, florescent Sertoli cells in a rat host that had not received immunosuppression therapy with cyclosporine A (CsA), and (B) shows viable, florescent Sertoli cells in the rat host that had received cyclosporine A immunosuppression therapy.

#### DETAILED DESCRIPTION OF THE INVENTION

Generally, the present invention provides a method for promoting the repair, protection, and support of dysfunctional tissue by mechanisms including *in situ* production of Sertoli cell-derived growth and regulatory factors referred to

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generally as trophic factors. Additionally, the present method provides a method of generating *in situ* trophic factor production. This is achieved by transplanting isolated Sertoli cells into a  
5 mammal, the cells secreting trophic factors *in situ*.

One significant benefit of utilizing Sertoli cells as an *in situ* factory for producing trophic factors is that Sertoli cells have been  
10 shown to have an effective immunosuppressant effect. Accordingly, concomitant adjunctive therapy to produce immunosuppression is not required. In other words, the Sertoli cells can be used as a trophic factor source while also  
15 providing a self-induced local immunosuppressive effect.

Trophic factors secreted by Sertoli cells include Sertoli cell-derived growth and regulatory factors such as insulin-like growth factors I and  
20 II, epidermal growth factor, transforming growth factors  $\alpha$  and  $\beta$ , and interleukin  $1\alpha$  (Griswold, 1992). For a more extensive list of Sertoli cell secretory factors refer to Table 1. Such factors have been shown to have an ameliorative effect on  
25 behavioral and functional deficits associated with neurodegenerative diseases. These factors are well

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known tropic factors which support normal cell and tissue metabolism and function. (Griswold, 1992). The present invention utilized the phenomenon that Sertoli cells can produce a trophic-rich, growth-  
5 supportive fluid microenvironment at the site of cellular dysfunction or cellular/tissue damage. Cellular/tissue damage can include, but is not limited to, radiation damage, burns and wounds. In contrast to the Sertoli cell/islet cell  
10 transplantation protocol used in the diabetic model, the method of the present invention utilizes only one type of cell, i.e. Sertoli cells, thereby significantly reducing the logistic and procedural problems inherent in attempting to transplant two  
15 different cell types at one host site.

Although rat Sertoli cells are utilized in the following examples, Sertoli cells from any suitable source can be used. For example, human Sertoli cells may be used for transplantation in  
20 humans. Additionally, in a preferred embodiment of the present invention, porcine Sertoli cells may be transplanted into a mammal, such as a human. Furthermore, veterinary uses of the present invention are contemplated and allogenic Sertoli  
25 cells would be selected for transplantation into the desired mammalian host.

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As demonstrated in the experimental section below, the present invention can be utilized as a treatment for ameliorating the behavioral and functional deficits associated with neurodegenerative diseases, such as Huntington's disease and Parkinson's disease. This can be accomplished without the concomitant side effects of previously utilized immunosuppressive adjuvant therapy, such as the chronic use of cyclosporine A.

5 The Sertoli cells, to provide both the secretion of the trophic factors and the immunosuppressive effect.

10

As shown in the examples below, the transplantation of Sertoli cells prior to inducing or formation of a brain lesion can provide a neuroprotective effect. For example, as demonstrated below, implantation of Sertoli cells prior to inducement of a Huntington's type disease provided both neuroprotective and prophylactic effects on a subsequent brain lesion. Therefore, the implantation of Sertoli cells early on following diagnosis of a neurodegenerative disease may provide useful treatment, prevention or reduction of the disease. Additionally, Sertoli cells may be transplanted in other types of CNS trauma such as head injury to treat, prevent,

15

20

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and/or prophylactically reduce the effects of CNS injury.

The following example demonstrates the ability of the present invention to ameliorate behavioral deficits associated with neurodegenerative disorders.

**EXAMPLE 1: SERTOLI CELL TRANSPLANTATION**

Specific Protocol:

10           The protocol generally involves two basic steps, (1) Sertoli cell isolation and (2) cell transplantation both of which are briefly described below (for greater details regarding the cell isolation see Selawry and Cameron (1993) and for  
15 details regarding cell transplantation, see Pakzaban et al. (1993) both incorporated by reference.

(1A) Sertoli Cell Isolation

20           The isolation procedure follows a well defined method Selawry and Cameron, (1993) and is routinely utilized. The cell culture medium used in all isolation steps and in which the cells were incubated was DMEM:Hams F12 supplemented with retinol, ITS, and gentamicin sulfate (Cameron and  
25 Muffly, 1991). Testes were surgically collected from sixteen day old male Sprague-Dawley rats. The

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testes were decapsulated and prepared for enzymatic digestion to separate other testicular cell types from the Sertoli cells. The enzymatic procedure utilized collagenase (0.1%), hyaluronidase (0.1%),  
5 and trypsin (0.25%) which is a typical procedure used in many cell isolation protocols. After sequential enzymatic digestion, the Sertoli cell isolate was washed with culture medium, transferred to sterile culture vessels and placed in a  
10 humidified, 5% CO<sub>2</sub> - 95% air tissue culture incubator. Following forty-eight hours of pre-incubation in a 39°C incubator, the Sertoli cells were washed to remove any contaminating debris. The resultant Sertoli cell-enriched fraction was  
15 resuspended into 0.25ml of DMEM/F12 medium and incubated at 37°C for at least 24 hours.

The Sertoli cells are then liberated from the vessel floor with trypsin, transferred to a sterile conical test tube, and repeatedly washed by  
20 centrifugation and treated with trypsin inhibitor to cease the enzymatic action of the trypsin. During the day of transplantation, the Sertoli cell-enriched fraction is resuspended and suctioned using a Hamilton syringe with a 20 gauge spinal  
25 needle.

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(1B) Isolation and Pretreatment of Sertoli Cells

Alternatively, as previously described (Cameron et al. 1987a; Cameron et al. 1987b)

5 decapsulated rat testes were subjected to sequential enzymatic treatment at 37°C using 0.25% trypsin (Sigma) and 0.1% collagenase (Sigma, type V) (Cameron et al. 1987a; Cameron et al. 1987b). The resulting Sertoli cell aggregates were equally

10 distributed in a volume of 20ml incubation medium into 75cm<sup>2</sup> tissue culture flasks (Costar). Plated Sertoli aggregates were incubated at 39°C in 5% CO<sub>2</sub>-95% air for 48 hours after which cells were subjected to hypotonic treatment with sterile 0.5mM

15 Tris-Hcl buffer for one minute (Galdieri et al. 1981) to expedite the removal of contaminating germ cells. Following two washes with incubation medium, flasks were replenished with 20ml incubation medium and returned to the CO<sub>2</sub>-injected

20 incubator at 37°C in 5% CO<sub>2</sub>-95% air. The resulting pre-treated Sertoli-enriched monocultures contained greater than 95% Sertoli cells. Plating density (< 2.0 X 10<sup>6</sup> Sertoli cells/cm<sup>2</sup>) did not result in a confluent monolayer of cells.

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## (2) Cell Transplantation

The transplantation protocol follows the procedure as previously described (Pakzaban et al., 1993). Animal surgery was carried out under  
5 sterile conditions. All animals were initially anesthetized with 0.60 ml/kg sodium pentobarbital and then were placed in a Koph stereotaxic instrument. Unilateral striatal transplants were performed using coordinates set at: anteroposterior  
10 = +1.2, mediolateral = +/- 2.8, dorsoventral = 6.0, 5.9, and 5.8 (based on the atlas of Paxinos and Watson, 1984). The striatum ipsilateral to the lesioned substantia nigra was transplanted with Sertoli cells. Each striatum receives a total  
15 volume of 3  $\mu$ l of Sertoli cell suspension. One microliter of the Sertoli cell suspension was infused over one minute per dorsoventral site. Controls only received media. Another five minutes was allowed upon reaching the last dorsoventral  
20 site before retracting the needle. After surgery, the animals were placed on heating pads to recover. Animals receive a short course of immunosuppression using Cyclosporine-A (20 mg/kg/d, i.p.) immediately after surgery and on the day following transplant.



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However, subsequent studies demonstrated that this short course of Cyclosporine-A is not needed (Figure 5A-B)

Sertoli cells are transplanted into  
5 animal models of various neurodegenerative disorders by stereotaxic coordinates defined for the specific disorder, as illustrated in the Parkinson's disease example, and then are systemically assayed for functional recovery by  
10 techniques specific to that animal model.

The present study used Sprague-Dawley male, eight week old rats with 6-OHDA-induced hemiparkinsonism (n=12). At three weeks post-lesion, the animals were subjected to behavioral  
15 tests that included the apomorphine-induced rotational behavior and the swing behavior. Baseline data showed significant apomorphine-induced rotational behavior (contralateral to the lesioned side of the CNS) in all these animals (at  
20 least 200 turns for 30 minutes). Using the elevated body swing test (EBST), significant right-biased swing activity (more than 70%) was also noted.

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At three weeks post-lesion, one group of animals (n=6) received Sertoli cells and one group (n=6) was subjected to the same surgical procedure but only received media (DMEM without serum) as  
5 controls. All animals received cyclosporine (20mg/kg) for the first two days following the transplant. At one, one and a half, and two months post-transplant, animals were again introduced in the same behavioral tests.

10 The animals receiving Sertoli cells exhibited significant reductions in rotations (mean of 50 turns for 30 minutes) while the animals receiving the media alone were at pre-transplant rotational level (Figure 1). The normalization of  
15 turning behavior persisted across the two month test period. The right-biased swing activity previously displayed by the Sertoli cells transplanted animals was also significantly reduced at post-transplant test sessions (Figure 2). The  
20 animals receiving the media did not show any significant reductions in their right-biased swing responses.

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At autopsy, brains were removed from the animals and fixed for vibratome sectioning at 40-80 $\mu$ m. Following staining, there was a marked reduction of activated glial cells at the

5 penetration site (i.e., lesion site) in Sertoli cell transplanted rats when compared to the penetration site in the lesioned animals not transplanted with Sertoli cells.

10 **EXAMPLE 2: GROWTH OF NEURAL CELLS**

Incubation medium and Sertoli cell pre-conditioned medium

The incubation medium used for Sertoli cell culture and co-culture was Dulbecco's Minimum

15 Essential Medium:Hams F12 Nutrient Medium (Whittaker Bioproducts) mixed 1:1 and supplemented with 3mg/ml L-glutamine (Sigma, grade III), 0.01cc/ml insulin-transferrin-selenium (ITS, Collaborative Research, Inc.), 50 ng/ml retinol

20 (Sigma), 19 $\mu$ l/ml lactic acid (Sigma) and 0.01cc/ml gentamicin sulfate (Gibco).

Following the first 48 hour incubation period of isolated Sertoli cells, media was collected and centrifuged at 1500rpm for 5 minutes.

25 The supernatant was collected and immediately

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frozen in sterile test tubes. This medium was identified as Sertoli pre-conditioned medium (SCM).

Isolation and incubation of fetal brain cells

5 Fetal brain cells (FBC) were collected from the ventral mesencephalon of fetal rats (15-17 days gestation). The fetal brain tissue was suspended in medium and initially dispersed by passing it through a series of sequentially  
10 decreasing sized hypodermic needles (18-26 gauge). The resulting suspension was treated with 0.1% trypsin for five minutes and followed by 0.1% trypsin inhibitor for two minutes. The suspended FBC were washed (3X), resuspended in incubation  
15 medium and plated in poly-L-lysine-coated culture vessels.

Cells from the ventral mesencephalon of fetal rats (VM) were isolated and cultured for seven days in control medium (CM) or Sertoli cell  
20 pre-conditioned medium (SCM) as shown in Figure 3A. VM cells incubated in CM showed no evidence of cellular stimulation or differentiation. Referring to Figure 3B, VM cells incubated in SCM were highly stimulated. Figure 3C illustrates that at higher  
25 magnification, VM cells incubated in SCM show

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neurite outgrowth as a response to Sertoli cell secreted trophic factors.

**EXAMPLE 3: IDENTIFICATION OF SERTOLI CELLS**

5                   Incorporation of latex beads:

Sertoli cells were isolated and prepared for incubation as described. Prior to transplantation (approximately 12 hours), sterile 1 $\mu$ m latex beads (10 $\mu$ l/ml medium; Pelco, Tustin, CA) were added to the incubation medium. Sertoli cells rapidly phagocytosed the beads. Immediately prior to transplantation, the beaded Sertoli cells were washed (three times) and resuspended in 1ml of incubation medium.

15                   Referring to Figure 4A, Sertoli cells were transplanted into the striatum of the brain wherein the penetration tract (arrows) and the site of Sertoli cell transplantation are shown. At higher magnification as shown in Figure 4B, Sertoli cells (arrows) were easily identified because of the inclusion of 1 $\mu$  latex beads which were loaded into the Sertoli cells prior to transplantation.

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**EXAMPLE 4: EFFECTS OF CYCLOSPORINE A (CSA) ON THE  
SURVIVAL OF TRANSPLANTED SERTOLI CELLS**

Fluorescent cell labeling:

Immediately prior to transplantation

5 (approximately two hours), Sertoli cell  
monocultures were treated with CM-DiI fluorescent  
dye for cell tracking (100 $\mu$ l stock/ml medium;  
Molecular Probes, Inc., Eugene, OR) for seven  
minutes at 37°C and then placed at 4°C for an  
10 additional 15 minutes. Fluorescent "tagged" Sertoli  
cells were washed (3X) and resuspended in 1ml of  
incubation medium.

The effect of cyclosporine A on the  
survival of grafted Sertoli cells *in situ* was  
15 examined. Grafted Sertoli cells were labeled with  
a fluorescent tag (DiI) prior to transplantation  
into the striatum of the brain. The tissue was  
collected one month post-transplantation.  
Referring to Figure 5A, viable fluorescent Sertoli  
20 cells were seen in a rat host that had not received  
immunosuppression therapy with cyclosporine A.  
Referring to Figure 5B, viable fluorescent Sertoli  
cells are shown in a rat host that had not received  
cyclosporine A immunosuppression therapy. This  
25 example demonstrates that cyclosporine A is not

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necessary for the survival of Sertoli cells transplanted into the brain.

**EXAMPLE 5: PROPHYLACTIC EFFECTS OF SERTOLI CELLS**

5           The transplantation of Sertoli cells is neuroprotective when implanted prior to inducing brain lesions. This prophylactic effect of Sertoli cells was demonstrated in an animal model for Huntington's Disease (HD). This model is produced  
10 by the systemic administration of the mitochondrial inhibitor, 3-nitropropionic acid (3NP). It has been demonstrated by Sanberg and colleagues (Koutouzis et al. 1994; Borlongan et al. 1995) and others that the injection of 3NP causes specific  
15 lesions within the striatum which mimic the pathology seen in Huntington's disease.

          In the present experiment 8 rats were transplanted with rat Sertoli cells (as described previously) unilaterally into one striatum of  
20 normal rats. Therefore, one side of the brain had Sertoli cells and the other side was without. One month later, the animals were injected with 3NP as described elsewhere (Koutouzis et al. 1994; Borlongan et al. 1995) to induce HD. Normal rats  
25 when injected with 3NP demonstrate bilateral damage of the striatum of the brain and have behavioral

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deficits which are equal on both sides of the body  
(Koutouzis et al. 1994; Borlongan et al. 1995).

One month following 3NP administration  
the animals demonstrated unilateral behavioral  
5 deficits. This was seen by the demonstration of  
apomorphine-induced rotations post-lesion in  
Sertoli transplanted animals, but not in controls  
(Number of Rotations; Controls=0.25±.6; Sertoli  
transplanted=197±31.9,  $p<.0001$ ). This asymmetric  
10 rotational behavior was indicative of a lesion on  
the side of the brain which was not transplanted  
with Sertoli cells. Therefore, Sertoli cell  
implants, vis-a-vis trophic mechanisms, have  
neuroprotective and prophylactic effects on  
15 subsequent brain lesions. This provides evidence  
that Sertoli transplantation may also be useful in  
treating neurodegenerative diseases early, before  
significant damage is present.

These results, taken together, show that  
20 the Sertoli cells ameliorate the behavioral and  
functional deficits of animal models of Parkinson's  
disease and Huntington's disease. The mechanism  
involved is most likely the secretion of Sertoli  
cell-derived growth factors, as demonstrated by the  
25 sprouting of neuronal tissue as shown in Example 2,  
and regulatory factors which promote the repair and



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the prolonged support of the relevant nervous tissue. Additionally, Sertoli cells may protect and promote nervous tissue repair in the brain by inhibiting glial cell activation at the lesion  
5 site. These results also demonstrate the viability *in situ* of transplanted Sertoli cells.

Throughout this application various publications are referenced by citation or number. Full citations for the publication are listed  
10 below. The disclosure of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15 The invention has been described in an illustrative manner, and it is to be understood the terminology used is intended to be in the nature of description rather than of limitation.

Obviously, many modifications and  
20 variations of the present invention are possible in light of the above teachings. Therefore, it is to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

TABLE 1

**I. Sertoli Cell-Derived Growth and Regulatory Factors (Partial List)**

<b>Category and Protein</b>	<b>Function</b>
<i>Hormones/Growth Factors</i>	
Mullerian Inhibiting Substance	inhibits Mullerian duct
Inhibin	inhibits FSH release
Insulin-like Growth Factor (Somatomedins A and C, IGF)	growth factor
Prodynorphin	
Interleukin-1 $\alpha$	mitogen
Transforming Growth Factor $\alpha$ & $\beta$	growth factors
Basic Fibroblast Growth Factor	growth factor
LHRH-like Factor	Leydig cell steroidogenesis
(unpurified or incompletely characterized)	
Sertoli Secreted Growth Factor	growth factor
Seminiferous Growth Factor	
Leydig Cell Stimulatory Activity	
Testins	
CMB proteins	
Vitamin Binding Proteins	vitamin transport
<i>Transport and Bioprotection</i>	
Transferrin	iron transport
Ceruloplasmin	copper transport
Saposin	binds glycosphingolipids
SGP-2 (Clusterin)	lipid transport?
Androgen Binding Protein	transports T and DHT
SPARC	calcium binding protein?
IGF Binding Proteins	IGF transport
Riboflavin Binding Protein	riboflavin transport
<i>Proteases and Protease Inhibitors</i>	
Plasminogen Activator	protease
Cyclic Protein-2	protease inhibitor
Cystatin	protease inhibitor
$\alpha_2$ -Macroglobulin	protease inhibitor
Type IV Collagenase	protease
Metalloproteinases	protease
<i>Basement membrane</i>	
Collagen IV	
Laminin	
Proteoglycans	

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**CLAIMS**

What is claimed is:

1. A method of generating in situ trophic factors by transplanting Sertoli cells into a tissue in need of trophic factors of a mammal, the cells creating trophic factors in situ.
2. A method as set forth in claim 1 wherein the tissue in need of trophic factors is the central nervous system of a mammal.
3. A method as set forth in claim 2 wherein the mammal suffers from a neurological disorder including a neural degeneration disorder, said method further including the step of ameliorating behavioral and functional deficits caused by the disorder by the action of the secreted trophic factors.
4. A method as set forth in claim 1, wherein the Sertoli cells are porcine Sertoli cells.
5. A method as set forth in claim 2, wherein said transplanting step is further defined as protecting the central nervous system from degenerative disorders.

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6. A method as set forth in claim 2 wherein said transplanting step is further defined as repairing damaged central nervous system tissue.

7. A method as set forth in claim 3, wherein the neurological disorder or neural degeneration disorder includes epilepsy, stroke, Huntington's disease, head injury, spinal injury, pain, Parkinson's disease, myelin deficiencies, neuromuscular disorders, neurological pain, amyotrophic lateral sclerosis, Alzheimer's disease, and affective disorders of the brain.

8. A method of generating *in situ* trophic factor production by transplanting porcine Sertoli cells into the central nervous system of a subject, the cells secreting trophic factors *in situ*, for treating neurological disorders including epilepsy, stroke, Huntington's disease, head injury, spinal injury, pain, Parkinson's disease, myelin deficiencies, neuromuscular disorders, neurological pain, amyotrophic lateral sclerosis, Alzheimer's disease, and affective disorders of the brain.

9. A method as set forth in claim 8, wherein the subject is human.

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10. A method of generating in situ trophic factor production by transplanting Sertoli cells into an area of tissue damage of a subject, the Sertoli cells secreting trophic factors in situ.

11. Use of Sertoli cells to generate in situ trophic factors by transplanting Sertoli cells into a tissue in need of trophic factors of a mammal, the cells creating trophic factors in situ.

12. A use as set forth in claim 1 wherein the tissue in need of trophic factors is the central nervous system of a mammal.

13. A use as set forth in claim 2 wherein the mammal suffers from a neurological disorder including a neural degeneration disorder, said use ameliorating behavioral and functional deficits caused by the disorder by the action of the secreted trophic factors.

14. A use as set forth in claim 1, wherein the Sertoli cells are porcine Sertoli cells.

15. A use as set forth in claim 2, wherein said transplanting is further defined as protecting the central nervous system from degenerative disorders.

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16. A use as set forth in claim 2 wherein said transplanting is further defined as repairing damaged central nervous system tissue.

17. A use as set forth in claim 3, wherein the neurological disorder or neural degeneration disorder includes epilepsy, stroke, Huntington's disease, head injury, spinal injury, pain, Parkinson's disease, myelin deficiencies, neuromuscular disorders, neurological pain, amyotrophic lateral sclerosis, Alzheimer's disease, and affective disorders of the brain.

18. Sertoli cells which are useful in generating *in situ* trophic factor production by transplanting porcine Sertoli cells into the central nervous system of a subject, the cells secreting trophic factors *in situ*, for treating neurological disorders including epilepsy, stroke, Huntington's disease, head injury, spinal injury, pain, Parkinson's disease, myelin deficiencies, neuromuscular disorders, neurological pain, amyotrophic lateral sclerosis, Alzheimer's disease, and affective disorders of the brain.

19. Sertoli cells which are useful in generating in situ trophic factor production as set forth in claim 18, wherein the subject is human.



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20. Sertoli cells which are useful in generating in situ trophic factor production by transplanting Sertoli cells into an area of tissue damage of a subject, the Sertoli cells secreting trophic factors in situ.

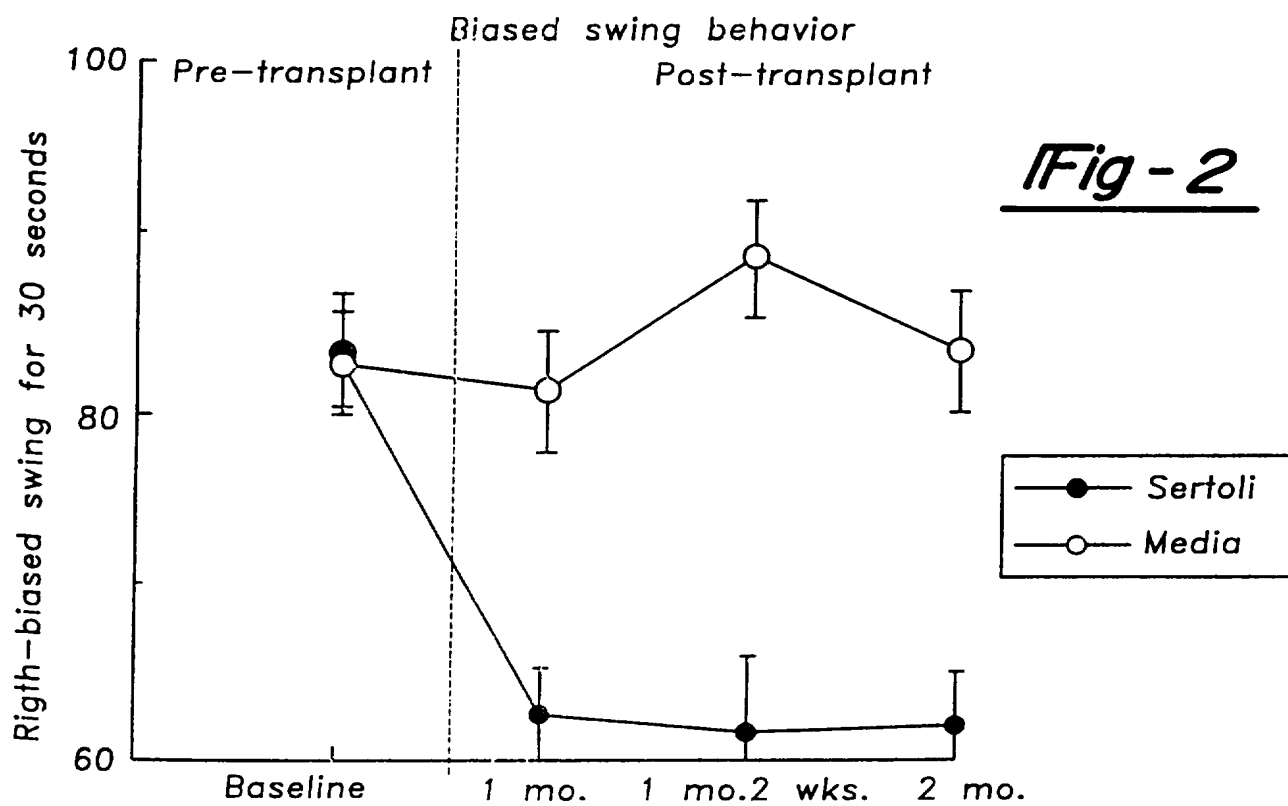
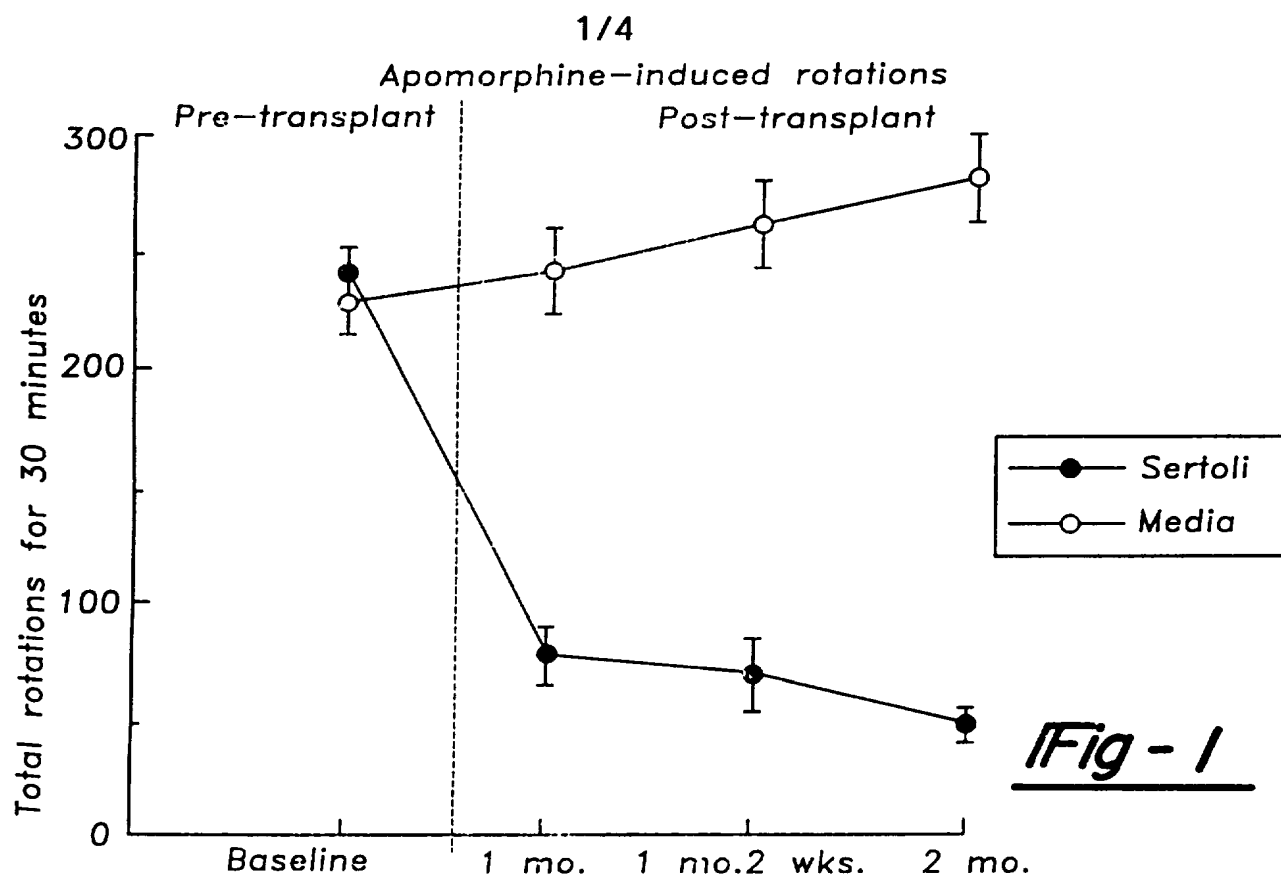


Fig - 3A



Fig - 3B

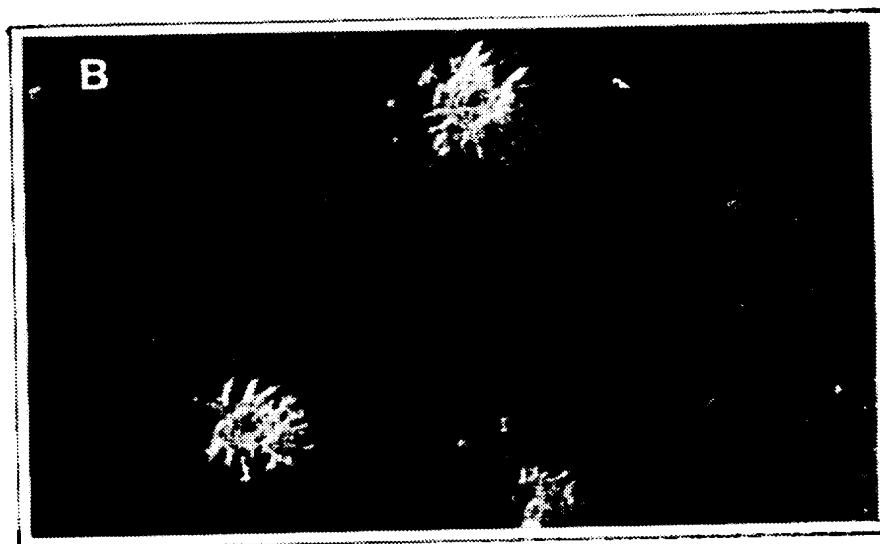


Fig - 3C



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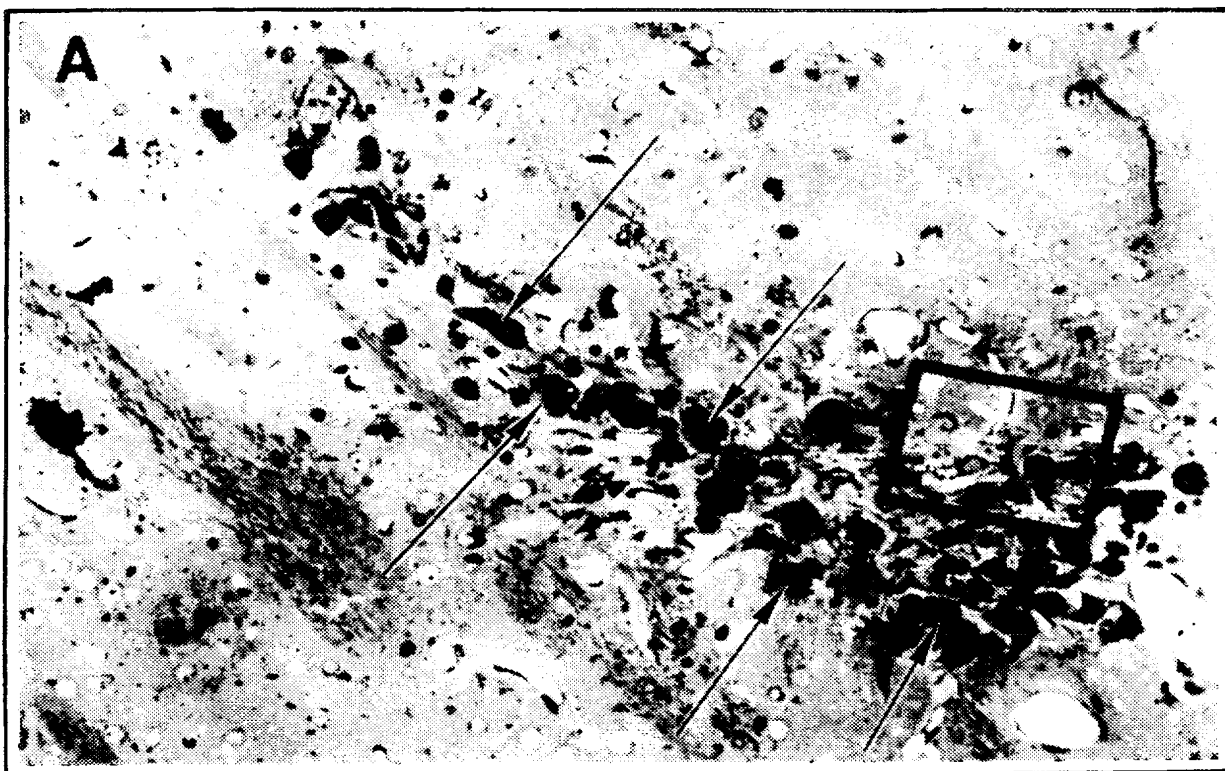


Fig - 4A



Fig - 4B

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Fig - 5A

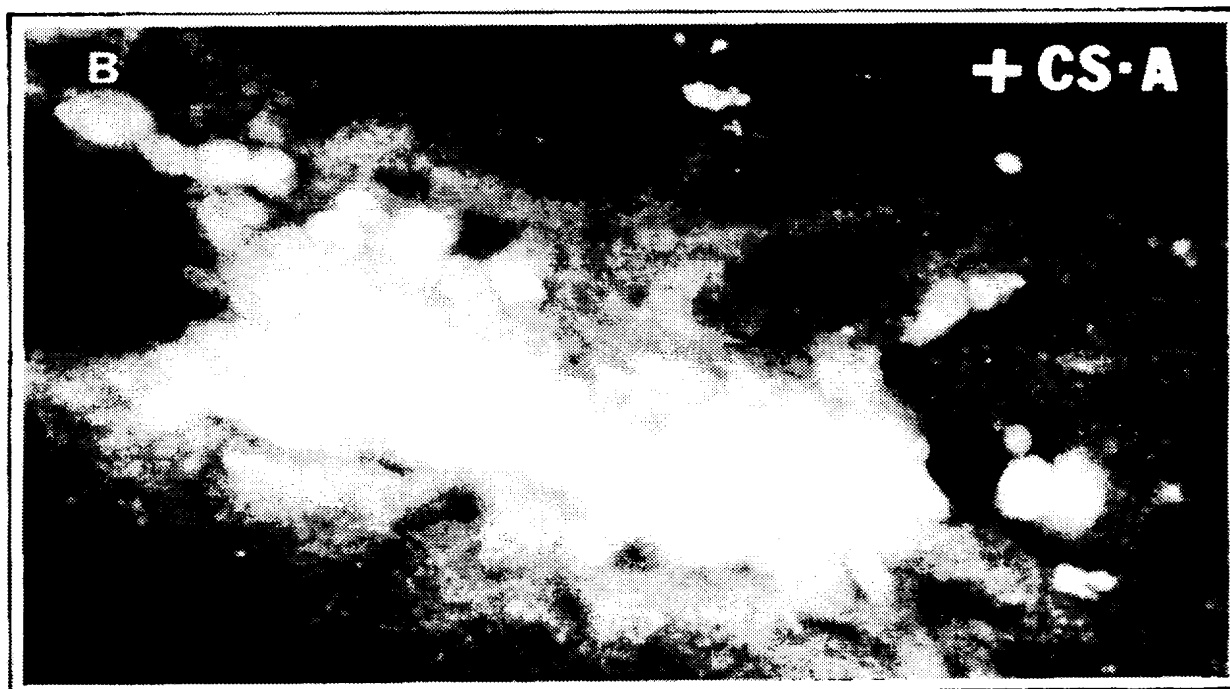


Fig - 5B

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/03335

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A01N 63/00; C12N 5/00

US CL : 424/93.7; 435/240.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.7; 435/240.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts, Biosis

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Lindvall et al. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's Disease. Science. 02 February 1990, Vol. 247, pages 574-577, especially pages 575-576.	1-17
X	Cameron et al. Hormonal regulation of spermatid binding. Journal of Cell Science. 1991, Vol. 100, pages 623-633, especially page 632.	1-17
X	Carson et al. Synthesis and secretion of a novel binding protein for retinol by a cell line derived from Sertoli Cells. Journal of Biological Chemistry, 10 March 1984, Vol. 259, pages 3117-3123, especially pages 3122-3123.	18-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

12 JUNE 1996

Date of mailing of the international search report

27 JUN 1996

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